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## (54) Reference electrode assembly

(57) Inter alia, a reference electrode assembly having a constrained-diffusion liquid junction between a liquid junction solution and a sample solution having separate flow paths, characterised in that it comprises:

(a) a flow cell having attached thereto a constraint comprising a region of porous material permeable

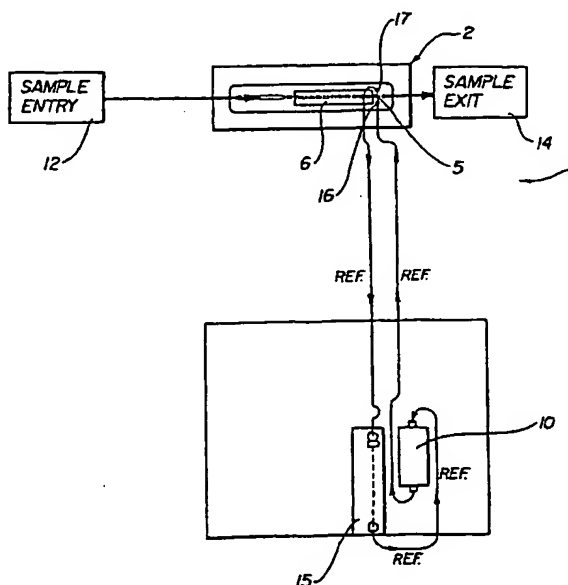
to water and salts;

(b) a remote reservoir for holding the said liquid junction solution;

(c) a means for moving the said liquid junction solution from the said reservoir to the said constraint; and

(d) a reference contact region; is disclosed.

FIG. 1



## Description

This invention provides a reference electrode device of the constrained-diffusion liquid junction type useful in pH and/or ion-selective electrode (ISE) potentiometric sensors and is particularly suitable for use in a mini-integrated electrochemical analyzer.

Conventional types of reference electrodes have a liquid junction where the sample meets the junction solution. The junction is typically either open or constrained. In an open junction system, the liquid junction operates by free diffusion. In a constrained-diffusion junction system, a region of porous material permeable to water and salts (a membrane, porous plug, frit, or the like) is placed at the site of the liquid junction. The porous material acts as a constraint whereby passage of large molecules (such as protein) and bulk liquid transport is generally hindered.

The liquid junction solution (also commonly referred to as the salt bridge solution) typically contains a solution saturated with a salt (such as an equitransferent salt, including KCl,  $\text{KNO}_3$ ) which functions to reduce and maintain constant the interfacial potential which develops across the liquid junction boundary, typically referred to as a liquid junction potential. The difference in liquid junction potentials between the system calibrator and the sample is referred to as the residual liquid junction potential. Typically, the residual liquid junction potential increases as the ionic strength difference between the system calibrator solution and the sample solution increases. The residual liquid junction potential is generally considered to compromise the accuracy of the associated potentiometric sensors and therefore a multi-use reference electrode is typically designed to minimize the residual liquid junction potential for as long as possible, while balancing other design constraints.

In potentiometric systems that are designed with miniaturized working electrodes (typically pH and/or ion selective electrodes), the necessity of the junction solution makes miniaturization of a reference electrode difficult. Further, for the reference electrode to have a multiple use capability, the liquid junction solution must be present in a volume and concentration to minimize the residual liquid junction potential over its useful lifetime. In order to minimize errors due to the residual liquid junction potential, the junction solution is generally saturated with the equitransferent electrolyte salt.

Conventional reference electrodes utilize a reference contact region (also sometimes referred to as electrode elements) immersed in a stagnant junction solution which contains a constant concentration of the equitransferent salt. The reference contact region is often silver based, consisting of an electrochemically reversible redox electrode couple such as  $\text{Ag}/\text{Ag}^+$  and  $\text{Ag}/\text{AgCl}$ . When salt solutions are used with silver based reference contact regions such as  $\text{Ag}/\text{AgCl}$ , the  $\text{AgCl}$  is susceptible to dissolution. In constrained-diffusion type liquid junctions, this dissolution is problematic because

it leads to subsequent precipitation of silver salts on the region of porous material constraint, thus leading to undesirable fouling of the constraint, which in turn generally results in an erratic reference electrode performance.

Commonly, because of the above-described fouling problem associated with the use of silver based reference contact regions, barrier membranes have been used to restrict  $\text{Ag}^+$  ion migration from the reference contact region to the porous material constraint region. The use of a barrier membrane, however, carries with it inconvenience because the first use wet-up of the reference electrode is hindered by the barrier membrane, thus requiring a long soaking time in the junction solution prior to the first use.

Another common problem associated with the use of saturated equitransferent salt solutions in constrained-diffusion type of liquid junction reference electrodes is that when the reference electrode is stored and/or used at sub-ambient temperatures, salt crystallization and precipitation may occur between the reference contact region and the region of the porous material at the junction, which in turn leads to erratic reference electrode potentials. An additional problem associated with the use of a saturated equitransferent salt solution is that the saturated solution may contribute to reproducibility and/or accuracy problems with blood samples because of interference caused by precipitation of protein and crenation of red blood cells present in the sample.

Reservoirs for holding of the junction solutions have been described for open (free diffusion) junction type of reference electrodes, where there is no region of porous material to act as a constraint at the junction. For example, A.K. Covington et al, (*Anal. Chim. Acta*, 1985, 169, pp. 221-229) describe a open junction where the junction solution is moved from a KCl reservoir via a syringe. In this prior art system, the liquid junction is established with each sample, but because this type of system leads to cross-contamination of the liquids upon use, the junction solution must be discarded along with the sample thus leading to increased waste.

Other prior art open free-diffusion liquid junction reference electrodes are gravity fed and thus require specific orientations and geometries to provide good reproducible junctions. (See, e.g., R.E. Dohner et al. *Anal. Chem.*, 1986, 58, pp. 2585-2589; T.R. Harbinson et al., *Anal. Chem.*, 1987, 59, pp. 2450-2456). The orientation and geometries requirements are particularly limiting when attempting to adapt such a reference electrode to a miniaturized system.

Thus, although many different reference electrodes are known in the prior art, there is a need to discover alternative reference electrodes for electrochemical analyzers, particularly reference electrodes that may be easily adapted for use in a mini-integrated type of analyzer.

Many of the problems associated with prior art ref-

erence electrodes have been solved with the discovery of a reference electrode assembly of the present invention. According to the invention, provided is a reference electrode assembly having a constrained-diffusion liquid junction between a liquid junction solution and a sample solution having separate flow paths, said assembly comprising (a) a flow cell having attached thereto a constraint comprising a region of porous material permeable to water and salts; (b) a remote reservoir for holding said liquid junction solution; (c) a means for moving said liquid junction solution from said reservoir to said constraint; and (d) a reference contact region. Also provided is a method for providing a reference for a potentiometric sensor analyzer, the method comprising moving a sample solution over at least one indicator electrode; moving said sample solution to a reference electrode flow cell to form a constrained-diffusion liquid junction between said sample solution and a liquid junction solution over a porous constraint wherein said liquid junction solution comprises a non-saturated equitransferent salt and is stored in a remote reservoir wherein said solution is pumped to said constraint per sample cycle and said liquid junction is electrically connected with sensing equipment by a reference contact region; and measuring an electric potential developed between said liquid junction solution and said indicator electrode.

The inventive reference electrode assembly is particularly useful because it is not orientation and gravity specific. Also, the assembly may be designed as a multiple use reference electrode for a mini-integrated electrochemical analyzer having planar miniaturized indicator electrodes because sufficient liquid junction solution is present for multiple uses without presenting a space problem because the extra liquid junction solution is stored in the reservoir that is remote from where the constrained-diffusion liquid junction is created.

As described in more detail in the Examples below, FIG. 1 is an embodiment of a reference electrode assembly. FIG. 2 shows a top view of a reference electrode.

According to the invention, the reference electrode has a flow cell where the liquid junction solution meets the sample solution at a junction constrained by a region of porous material permeable to water and salts. The porous material generally hinders passage of large molecules (such as protein) and bulk liquid transport. Such porous materials have been used extensively in the art as the "constraint" in constrained-diffusion liquid junction reference electrodes and thus are easily recognizable by those skilled in the art and are widely commercially available. Examples of such materials are porous plugs, frits and/or membranes. When selected as the constraint material, porous membranes may be fashioned from such materials as cellophane, cellulose acetate, partially nitrated cellulose polycarbonate, combinations thereof, and so on. Particularly preferred, because of ease of use, cost and availability, is a cellophane membrane.

The flow cell may be fashioned from any suitable material conducive to the overall design of the analyzer such that the flow cell material is capable of attachment to the constraint material, as well recognized by those skilled in the art. If the reference contact region is configured as a part of the flow cell, then the material selected should be non-conductive. If the reference contact region of the reference electrode assembly is not a portion of the flow cell, then the material selected may be conductive, if desired.

Attaching the constraint region of porous material to the flow cell where the junction is formed may be accomplished by any number of methods within the skill of one acquainted with the art, including for example, bonding or attaching with welding, adhesives, mechanical compression and the like. The attachment should be secure enough to substantially prevent regions of dead volume in the junction solution. Preferably a hermetic seal is formed between the flow cell and the constraint material. The area of the porous material exposed to the sample and/or the junction solution may vary, spanning from the entire flow cell to only a small portion of the flow cell, depending upon the specifications of the analyzer design, on so on. The flow cell may optionally be designed such that the porous material covers a distinct chamber region of the flow cell, where the constraint covers the chamber region and the liquid junction is formed over the chamber region.

The remote reservoir aspect of the invention functions as a storage unit for excess junction solution. According to the invention, the reservoir is external to the constrained-diffusion region where the liquid junction is formed. The external nature of the reservoir allows for a multiple use reference electrode without requiring a bulky storage unit for the junction solution at the liquid junction itself. The reservoir may be designed in various sizes and shapes, depending upon the protocol of the overall analyzer system and the desired lifetime of the reference electrode assembly. For example, the reservoir could be formed by holding the junction solution in the typical tubing (or tubing with a bladder) leading to the region of porous material where the liquid junction is formed. Alternatively, the reservoir may be recognized as a separate container connected by tubing or other such means to the constrained-diffusion liquid junction. Appropriate separate structures that may be used as the reservoir include bottles or tanks and so on.

The liquid junction solution comprises an aqueous solution of a salt having equivalent cationic and anionic conductances, as are well known in the art and include equitransferent salts, such as KCl, KNO<sub>3</sub> and equivalents thereof. Non-equitransferent salts, such as NaCl, NaBr, KBr, NaNO<sub>3</sub> equivalents thereof and so on may also be used, however, these lead to increased liquid junction potentials. Preferred are equitransferent salts (KCl, KNO<sub>3</sub>, and mixtures thereof). Because of cost and availability KCl is the most preferred salt. Other items may optionally be present in the liquid junction solution,

including, for example, compatible surfactants, or ions.

The use of silver ions in the liquid junction solution is useful in the embodiments where the reference contact region is a silver based material. Preferably, when the electrical contact region does not have a barrier membrane and the salt solution (preferably KCl) is non-saturated, then the junction solution further comprises  $\text{Ag}^+$  ions, the ions preferably present in an amount sufficient to establish stable potentials at the silver based reference contact region but low enough so as to not induce fouling at the porous material constraint. It is known from the prior art that the concentration of  $\text{Ag}^+$  ions present at saturation is dependent on the concentration of the electrolyte salt present in the junction solution (e.g., see Forbes, G.S., *Journal of the American Chemical Society*, **33**, pp. 193746 (1911), hereby incorporated by reference). Preferably, the  $\text{Ag}^+$  ions are included in the junction solution to the point of saturation when the KCl solution is used at a concentration of less than about 2 M. Preferably when the KCl is present in a concentration of greater than about 2 M to saturation (4.2 M at room temperature or 7.1 M at 100°C), then the  $\text{Ag}^+$  ions are provided in a concentration below saturation from about 0.01 mM to about 1 mM, most preferably 0.6 mM. The  $\text{Ag}^+$  ions may be provided to the salt solution or formed in conjunction with the preparation of the junction solution by any number of familiar methods. For example, the junction solution may be prepared from a dilution of a saturated KCl/saturated AgCl solution; addition of solid KCl to a 2 M KCl/saturated AgCl solution; dissolution of appropriate quantities of KCl and  $\text{AgNO}_3$  in water, and so on.

Any desired concentration of the equitransferent salt in the junction solution may be utilized. Although saturated solutions of the salt may be used, one of the advantages of the inventive assembly is that the salt may be used in a concentration below saturation. In a preferred embodiment, a non-saturated solution of the salt is employed. Preferably KCl is selected as the salt and used in an amount ranging from about 0.5 M to about 7.1 M, more preferably 1 M to 4.5 M, and most preferably about 3.5 M. One of the advantages in using a non-saturated solution is particularly evident when a Ag/AgCl material is used as the reference contact region because with the non-saturated KCl solution, the  $\text{Ag}^+$  concentration present at saturation is lowered (thus assisting in minimizing AgCl dissolution).

The means for moving the liquid junction solution from the reservoir to the constraint region may be selected from various known devices, taking into effect the overall design of the analyzer. Included in the definition of devices that may be selected for the means for actively moving the liquid are devices such as pumps, syringes, and the sort. For automated systems, a peristaltic pump, reciprocating syringe or other such device is particularly useful.

The design of the analyzer may be such that the device used for movement of the junction solution may

also be used for moving other liquid(s) in the analyzer, such as, for example, the sample solution, calibration solution, wash solution, combinations thereof, and so on. Alternatively, a device specific to movement of the liquid junction solution alone may be used. The flow rate and volume of the liquid junction solution may be adjusted to the specifications of the analyzer to provide a concentration of electrolyte salt at the liquid junction that resembles that of the salt solution of the reservoir, as recognizable to those skilled in the art.

The reference contact region may be placed anywhere within the analyzer as long as it may function so that electrical contact is made between the sensing equipment and the liquid junction solution. Suitable materials for the reference contact region are materials including conductive metals, alloys thereof, and composites thereof having acceptable conductive properties, as are easily recognized by those skilled in the art (i.e. materials that are capable of providing an electrochemically reversible redox electrode couple). Particularly suitable are silver based materials and calomel ( $\text{Hg}/\text{Hg}_2\text{Cl}_2$ ). More preferably silver based materials are used (e.g., Ag/ $\text{Ag}^+$  and Ag/AgCl). Although barrier membranes covering all or a portion of the electrical contact regions may be used, for ease of use, preferably no such barrier membrane is present in the reference electrode.

For ease of use, the reference contact region may be designed into multiple conductive regions that are connected together to facilitate electrical contact between the sensing equipment and the liquid junction. For example, two regions may be used. In this design, the first region is where the contact is made with the liquid junction solution, with the first region prepared from materials that are capable of providing an electrochemically reversible redox electrode couple. The second region is directly connected to the first region but does not necessarily touch the liquid junction and may be prepared using any suitable electrically conductive materials (i.e. extending beyond those materials capable of providing an electrochemically reversible redox electrode couple). In this design, the function of the second region is to facilitate making electrical contact to the sensing equipment. Illustrative of this set up would be a first region comprising a conductive wire connected to the liquid junction solution and a second region that is a "pad" embedded or screen printed onto the non-conductive flow cell. In this embodiment the wire would be set into the pad in a perpendicular fashion. The electrical contact(s) from the sensing equipment would touch the second region pad, thus providing indirect contact between the sensing equipment and the liquid junction solution.

The reference electrode assembly may be used in conjunction with one or more potentiometric indicator working electrode(s) whose response depends upon analyte concentration. Ion-selective electrodes based on solvent polymeric membranes are examples of such indicator electrodes. Examples of analytes that may be measured include, for example, chloride, potassium,

calcium, sodium, pH, bicarbonate, magnesium, and so on. The reference electrode and the indicator electrode constitute individual galvanic half-cells which together comprise an electrochemical cell which allow for potentiometric quantitative analysis. The reference electrode assembly is capable of providing a steady and stable potential sufficient for potentiometric analysis over the clinically relevant range of ionic strengths, protein concentrations, and hematocrit levels. Optionally, the reference electrode assembly may be packaged together with mini-integrated working electrodes stationed on a sensor module such that the entire configuration is a single disposable unit.

The following Examples are intended to illustrate the invention.

### Examples

Many configurations of the reference electrode assembly may be designed. FIGs. 1-2 illustrate a configuration of a reference electrode assembly incorporated into a mini-integrated electrochemical analyzer 1 where the reference electrode has a lifetime of at least about one month with typical clinical usage.

More particularly, in FIG. 1 the schematic view illustrates a reference electrode 5 positioned behind an array of a plurality of working electrodes 6. The junction solution is pumped from a remote external reservoir 10 located apart from the liquid junction region of the reference electrode 5. The orientation of the reservoir 10 is not gravity specific, and thus the reservoir may be placed anywhere that is convenient to the other items of the analyzer. From the sample entry 12 portion of the sensor array, the analyte containing sample is pumped through the various working electrodes to a sample exit 14. The sample solution flow path and liquid junction solution flow path are separate paths. A common pump 15 is used for sample solution movement and junction solution movement. The junction solution is moved from the remote reservoir 10 to the site of the reference electrode 5 each time a sample is run. The movement of the junction solution may be continuous but more preferably occurs only at such time as when a sample (or reference liquid such as a calibrator or quality control material) is present. Once used, the junction solution may be discarded as waste or recycled and pumped back to the reservoir 10. Preferably the solution is recycled, thus extending the lifetime of the reference electrode and also eliminating waste. In a particularly preferred configuration, the liquid junction solution is re-circulated and pumped with the introduction of each analyte sample and becomes stagnant after the testing of the sample is completed. Preferably, only a small volume of the reference electrolyte solution is moved to the constraint region of the reference electrode 5 and optionally recycled back to the external reservoir 10 per cycle, thus allowing for a small front-end reference electrode assembly particularly useful in a mini-integrated analyzer.

As shown in FIGs. 1 and 2, the liquid junction solution may be pumped from the reservoir 10 to an inlet tube 16 over the region of porous material forming the constraint 18. The porous material (preferably a permeable membrane) is attached to the flow cell by fastening a gasket over the material whereby a hermetic seal between the porous material and the flow cell is formed. The area constrained by the porous material is where the sample and the junction solution interface in the flow cell thus forming the liquid junction. The flow cell has a defined elliptical shaped chamber, where the shape of the chamber helps provide an effective washout of the entire chamber and also minimizes bubble trapping. Junction solution depletion occurs at a rapid rate once the junction solution is held stagnant in the flow cell. Design of the surface areas and volume of the elliptical chamber relative to the sample volume and hold time may be manipulated to provide the desired performance. Preferably when such an elliptical shaped chamber is used, the constraint region is attached thereto. In a preferred embodiment, the elliptical chamber has a volume of from about 3.5 to about 1.7  $\mu\text{L}$ , most preferably about 3.5  $\mu\text{L}$ . The electrical contact region has two parts. The first region 11 is a  $\text{Ag}^\circ$  or  $\text{Ag}/\text{AgCl}$  wire that is imbedded in a second contact region 25 that is attached to the non-conductive flow cell. The second region 25 has an exposed portion in the flow cell through which the electrical contact is made with the sensing equipment. The sample is removed from the sensor array through the sample exit 14 as waste while the junction solution is recycled back to the reservoir 10 via the exit tube 17.

In the embodiment shown in FIGs. 1 and 2, the reference electrode most preferably employs a non-saturated KCl liquid junction solution stored in a reservoir having a volume of from about 1 mL to about 50 mL (most preferably 8 mL), a membrane porous constraint region, and a silver based reference contact region without a barrier type of membrane. Advantages of this embodiment include a multiple-use capability, with minimum fouling of the porous material region over the use lifetime. The assembly may be stored as a dry reference electrode assembly. The KCl junction solution may be released from the reservoir which then easily wets the flow cell upon first use, where bubble formation at the membrane region (which typically accompanies a first use) is reduced. Also, in this preferred embodiment, the junction solution is re-circulated and thereby greatly minimizes the reservoir volume necessary for multiple use and also reduces the amount of waste generated as compared to systems with one-use only solutions. This reference electrode also may be stored at sub-ambient temperatures with a reduced possibility of salt crystallization while providing reproducible results having an acceptable accuracy with multiple testing capability. In this embodiment there is exhibited an adequate ionic strength independence at relatively low junction solution concentration, as low as about 0.5 M KCl. This

is unexpected based on the Henderson equation for the liquid junction potential of two freely diffusing liquids.

#### Example 1

Reference flow cells were either machined from rigid acrylic or injection molded using ABS plastic. For each flow cell constructed, the constraint material was a ion and water permeable free-standing 0.001" thick cellophane (uncoated regenerated cellulose) film membrane obtained from Flexel, Inc., Atlanta, GA. The film was placed over an elliptical shaped chamber of the flow cell (the chamber having a volume of approximately 3.5  $\mu$ L). The film was hermetically sealed to the chamber region of the flow cell with compression from a gasket on top of the flow injection sensor module assembly. Sample solution and liquid junction solution flowed past each other on opposite sides of the surface of the cellophane film membrane at a flow rates of from about 5 to about 100  $\mu$ L/sec. A common peristaltic pump was used to simultaneously move both solutions. Electrical contact was established via an electrochemically plated 0.012" Ag/AgCl or Ag wire located either within the remote reservoir solution or in the base of the flow cell imbedded in screen printed epoxy silver in a perpendicular fashion to extend into the inlet tube of the flow cell. The sensing equipment used was a HP 3457A digital multimeter. The total volume of the solution in the junction reservoir ranged from approx 8 mL to approx. 15 mL. The junction solution was moved from the remote reservoir to the flow cell and past one surface of the cellophane membrane with every sample solution. The junction solution was then either re-circulated back to the reservoir or sent to waste after the measurement cycle. Electrode measurements were taken when both the sample and liquid junction solutions were stationary.

Various junction solutions were tested. The aqueous based solutions tested were (a) 1.5 M of KCl saturated with AgCl (approx. 0.25 mM); (b) 2.0 M of KCl saturated with AgCl (approx. 0.6 mM); (c) 3.5 M of KCl with 0.6 mM of AgCl; and (d) 4.0 M of KCl with 0.6 mM of KCl, where some of the solutions further comprised about 0.05 g/L of BRIG® 700 (manufactured by ICI Surfactants, Wilibrington, Del.). The wet up of a fully dry system was established to commercially desirable specifications in less than five minutes. Ionic strength dependence over a clinically significant range of 0.120 mM to 0.200 mM was evaluated. Reference potential changes of less than 0.5 mV were measured for all solutions tested over a time period averaging approx. 1 month. This reference electrode was used together with separate planar ion-selective electrodes sensitive for pH, potassium, ionized calcium, and sodium for the analysis of these analytes.

#### Example 2

Reference electrodes were prepared as described

in Example 1 with the following exceptions.

The placement of the reference contact region (a Ag wire) was varied. A three dimensional sodium ISE working electrode (200 Series obtained from Ciba Corning Diagnostics Corp., Medfield, MA) was tested with three reference electrodes using a junction solution of 2M KCl/saturated with AgCl. The reference electrode used as the control was a 200 Series Coming Diagnostics Corp three-dimensional type of reference electrode. The calibration reagents were that used on the Ciba Corning Diagnostics Corp. 644 Instrument.

In Set A, the reference electrodes tested had a Ag wire located in the base inside the elliptical chamber. A reference electrode prepared with a Ag wire in the base of the flow cell was tested over a period of 35 minutes. The average within sample drift was -0.047 mV/sec. A reference electrode with a Ag wire in the remote reservoir was tested under the same conditions and was found to have an average within sample drift of -0.001 mV/sec. The control 200 Series reference electrode had an average within sample drift of -0.0004 mV/sec.

In Set B, the reference electrodes tested had a Ag wire located in a base outside the elliptical chamber. A reference electrode prepared with a Ag wire in the base outside the elliptical chamber of the flow cell was tested over a period of 35 minutes. The average within sample drift was 0.001 mV/sec. A reference electrode with a Ag wire in the remote reservoir was tested under the same conditions and was found to have an average within sample drift of 0.0004 mV/sec. The control 200 Series reference electrode had an average within sample drift of 0.001 mV/sec.

#### Example 3

Reference electrodes were prepared as described in Example 1 with the exception that the elliptical chamber had a larger volume (16.8  $\mu$ L) as compared with the elliptical chamber of Example 1 (which had a volume of 3.5  $\mu$ L). A three dimensional sodium ISE working electrode (as described in Ex. 2) was tested with three reference electrodes using a junction solution of 2M KCl/saturated with AgCl. The reference electrode used as the control was a 200 Series Coming Diagnostics Corp three-dimensional type of reference electrode. The calibration reagents were that used on the Ciba Corning Diagnostics Corp. 644 Instrument. The reference electrodes tested had a Ag wire located in a base inside the elliptical chamber. The electrodes were tested over a period of 35 minutes. The average within the sample drift was -0.012 mV/sec. When repeated the average within the sample drift was -0.028 mV/sec. The control 200 Series reference electrode had an average within sample drift of 0.001 mV/sec.

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## Claims

1. A reference electrode assembly having a constrained-diffusion liquid junction between a liquid junction solution and a sample solution having separate flow paths, characterised in that it comprises:
  - (a) a flow cell having attached thereto a constraint comprising a region of porous material permeable to water and salts;
  - (b) a remote reservoir for holding the said liquid junction solution;
  - (c) a means for moving the said liquid junction solution from the said reservoir to the said constraint; and
  - (d) a reference contact region.
2. An assembly as claimed in claim 1 wherein the said constraint is a membrane, which is preferably cellophane attached to the said flow cell with a hermetic seal.
3. An assembly as claimed in claim 1 or claim 2 wherein the said liquid junction solution comprises an equitransferent salt solution, which is preferably present in a non-saturated concentration, the salt preferably being KCl.
4. An assembly as claimed in claim 3 wherein the said liquid junction solution further comprises  $\text{Ag}^+$  ions and the said reference contact region is a silver-based conductive material.
5. An assembly as claimed in claim 4 wherein the said  $\text{Ag}^+$  ions in the said liquid junction solution are present in an amount of from about 0.01 to about 1 mM and KCl is present in an amount of from about 1 M to about saturation, preferably the said  $\text{Ag}^+$  ions in the said liquid junction solution are present in an amount of about 0.6 mM and the said KCl is present in an amount of from about 2 to about 4 M.
6. An assembly as claimed in any of claims 1 to 5 wherein the said reference material consists essentially of bare Ag or Ag/AgCl; the said means for moving the said liquid junction solution is a pump; and the said liquid junction solution comprises an equitransferent salt present in a non-saturated concentration and  $\text{Ag}^+$  ions.
7. An assembly as claimed in any of claims 1 to 6 wherein the said liquid junction solution is recycled back to the said reservoir after functioning at the said liquid junction per sample measurement cycle.
8. An assembly as claimed in any of claims 1 to 7 wherein the volume of the said remote reservoir is from about 1 to about 50 mL, preferably about 8 mL

with a one month use life.

9. A reference electrode assembly having a constrained-diffusion liquid junction between a liquid junction solution and a sample solution having separate flow paths, characterised in that it comprises:
  - (a) a flow cell having a chamber region wherein a region of porous membrane constraint permeable to water and salts is attached to the said chamber region of the said flow cell;
  - (b) a remote reservoir for holding the said liquid junction solution comprising an equitransferent salt and  $\text{Ag}^+$  ions, the said reservoir being a separate container connected by tubing to the said region of porous membrane constraint;
  - (c) a pump for moving the said liquid junction solution from the said reservoir through the said tubing to the said constraint; and
  - (d) a reference contact region comprising a silver-based material.
10. A method of providing a reference for a system for potentiometric quantitative analysis characterised in that it comprises:
  - (a) moving a sample solution over at least one indicator electrode;
  - (b) moving the said sample solution to a reference electrode flow cell to form a constrained-diffusion liquid junction between the said sample solution and a liquid junction solution over a porous constraint, the said liquid junction solution comprising a non-saturated equitransferent salt and being stored in a remote reservoir, the said solution being pumped to the said constraint per sample cycle and the said liquid junction solution being electrically connected with sensing equipment by a reference contact region; and
  - (c) measuring an electric potential developed between the said reference contact region and the said indicator electrode.
11. A method as claimed in claim 10 wherein the said liquid junction solution is recycled after (b) to the said remote reservoir.
12. A method as claimed in claim 10 or claim 11 wherein the said liquid junction solution is stagnant until such time as (a) is performed.
13. A method as claimed in any of claims 10 to 12

wherein the said reference contact region is a silver-based conductive material and the said junction solution comprises (a) KCl present in an amount of about 3.5 M and (b)  $\text{Ag}^+$  ions present in an amount of about 0.6 mM.

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14. A method as claimed in any of claims 10 to 13 wherein the said indicator electrode is a planar miniaturized electrode.

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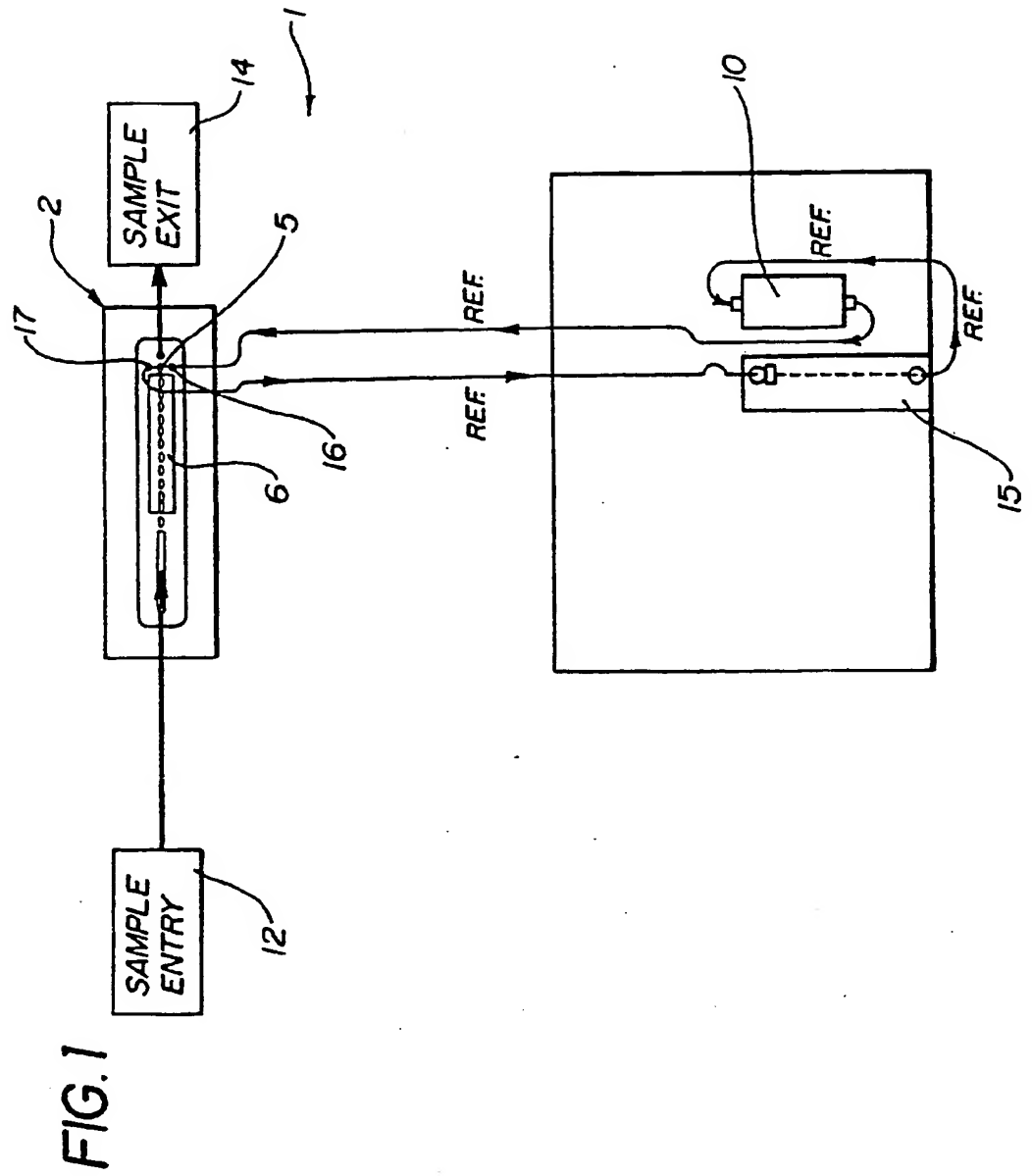
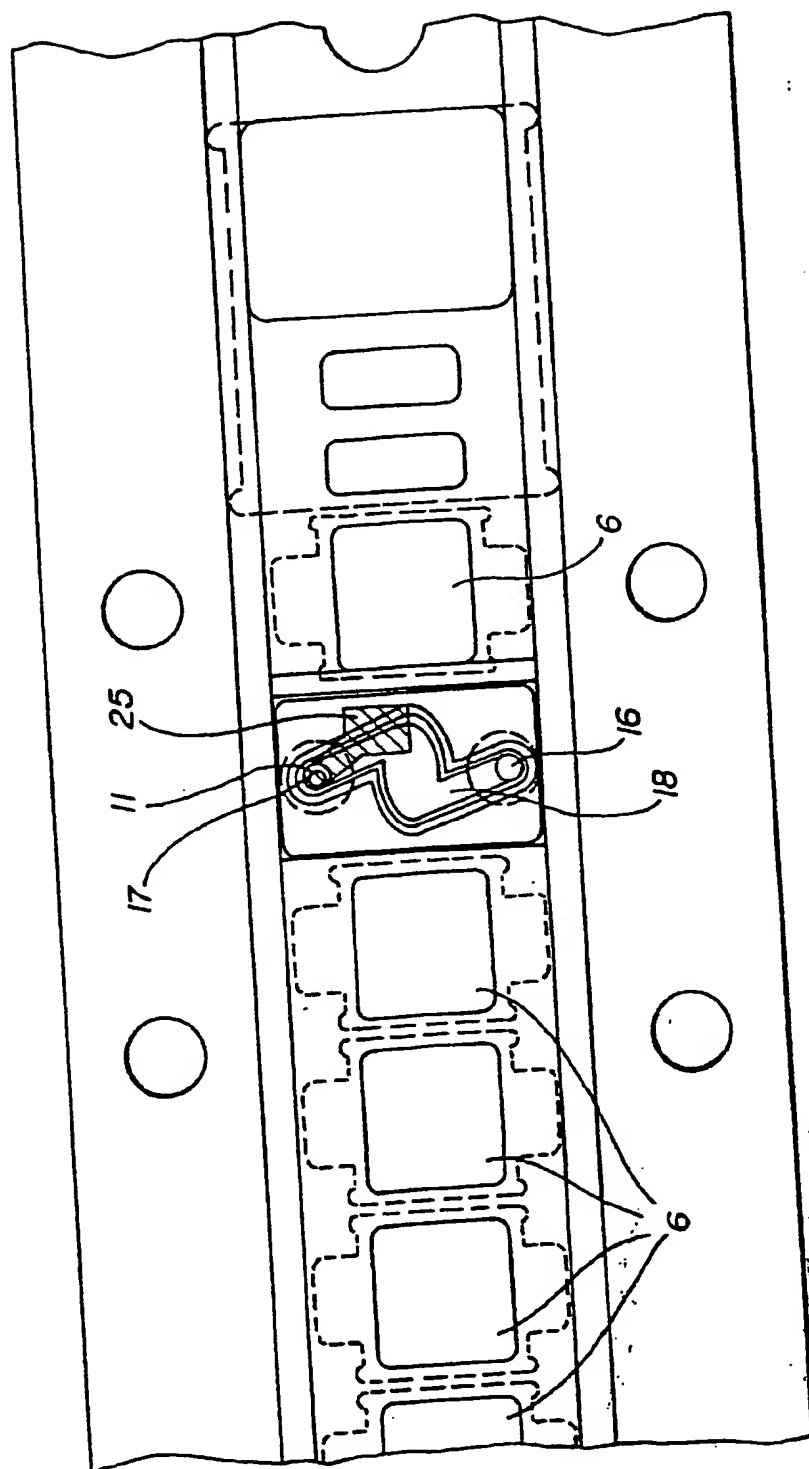


FIG.2





European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 96 30 7937

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	EP-A-0 388 017 (BECKMAN INSTRUMENTS, INC.) * abstract; figure 2 *	1	G01N27/28
Y	--- PATENT ABSTRACTS OF JAPAN vol. 6, no. 129 (P-128) [1007] , 30 March 1982 & JP-A-57 053648 (TOKYO SHIBAURA DENKI K. K.), 15 July 1982, * abstract *	1	
A	--- EP-A-0 201 712 (EPPENDORF GERÄTEBAU NETHELER + HINZ GMBH) * abstract; figure 1 *	1	
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 10 February 1997	Examiner Duchatellier, M
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document & : member of the same patent family, corresponding document	

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